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Mechanisms of coronary vasodilatation produced by ATP in guinea-pig isolated perfused heart

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1 Isolated hearts of guinea-pigs were perfused *in vitro* with a physiological salt solution via a retrograde aortic cannulation (Langendorff preparation) at constant perfusion pressure. Bolus intra-arterial injections of various vasodilator drugs were made and the coronary flow responses were measured with an electromagnetic flow probe placed in the arterial inflow circuit. Inhibitory drugs were infused intra-arterially.

2 Nitro-L-arginine (NLA; 500 μM), an NO synthesis inhibitor, decreased coronary baseline flow by 16 $\pm$ 0.8%, converted acetylcholine-induced coronary vasodilatation to vasoconstriction and had no effect on coronary flow responses to adenosine or papaverine. Sodium nitroprusside-induced responses were enhanced during NLA infusion by 46 $\pm$ 11%.

3 Adenosine 5'-triphosphate (ATP) increased coronary flow but coronary flow responses to ATP were not altered by infusion of NLA.

4 ATP-induced coronary dilatation was not significantly attenuated by infusion of the adenosine receptor antagonist XAC, (xanthine amine congener; 2 μM), whereas XAC decreased coronary flow responses to adenosine by 75% $\pm$ 5%.

5 ATP-induced coronary flow responses were reduced by only 31 $\pm$ 4% during indomethacin infusion (2.8 μM) whereas indomethacin completely eliminated the initial vasoconstriction phase and greatly attenuated the peak flow and duration of the later vasodilatation phase seen in response to arachidonic acid (0.75 nmol). Indomethacin had no effect on vasodilatations produced by adenosine or prostaglandin I_2.

6 These results indicate that ATP-induced coronary dilatation in the isolated, perfused heart of the guinea-pig is not dependent upon NO production or upon degradation of ATP to adenosine. The coronary dilator action of ATP may be partially dependent (~30%) upon the production of vasodilator prostaglandins.

Keywords: Nitric oxide; nitro-L-arginine; indomethacin; adenosine; xanthine amine congener; prostaglandins; coronary vasodilatation

Introduction

Vasodilatation induced by adenosine 5'-triphosphate (ATP) has been shown to be be wholly or partially endothelium-dependent in several types of isolated blood vessel (De Mey & Vanhoutte, 1981; Cassis *et al.*, 1987) and *in vivo* microcirculation preparations (Koller *et al.*, 1991), in which the endothelium is easily removed or damaged. The endothelium-dependence of the vascular actions of ATP has proved more difficult to evaluate in whole-organ preparations, such as the isolated heart, because of the problem of selectively damaging the endothelium in such preparations. A role for endothelium-derived relaxing factor (EDRF), thought to be nitric oxide (NO) or a nitrosyl compound, in ATP-induced coronary dilatation has been proposed. This suggestion is based upon the measurement of NO release from isolated hearts stimulated by ATP (Kelm & Schrader, 1990) and upon the inhibitory effects of hydroquinone (Hopwood *et al.*, 1989) and electrolytically generated oxygen radicals (Lee *et al.*, 1990) on the coronary response to ATP. Although hydroquinone and oxygen radicals are known to inactivate NO, the specificity of these interventions is uncertain. Thus, this evidence is not conclusive. ATP also causes the release of prostaglandin I_2 (PGI_2) from the heart (Needleman *et al.*, 1974), presumably in large part from the endothelium (Needham *et al.*, 1987). Therefore, two possible endothelium-derived mediators of ATP-induced coronary vasodilatation are NO and PGI_2. It has also been proposed that some of the vasodilatation produced by ATP is, in fact, due to its degradation to adenosine (Moody *et al.*, 1984).

This study was designed to test the hypothesis that ATP-induced coronary vasodilatation is mediated by NO, prostaglandins and adenosine. This was accomplished by use of specific inhibitory agents: nitro-L-arginine (an inhibitor of NO synthase), indomethacin (a cyclo-oxygenase inhibitor) and xanthine amine congener (XAC, an adenosine receptor antagonist).

Our results show that ATP-induced coronary vasodilatation in guinea-pig isolated heart is not mediated by NO or adenosine. A portion of the response (about one-third) may be due to the production of vasodilator prostaglandins.

Methods

Isolated heart preparation

Male guinea-pigs (275–375 g) were anaesthetized with sodium pentobarbitone (65 mg ml^{-1} or to effect, i.p.). Heparin (400 u) was injected intravenously. After the thorax was opened, the heart was quickly excised, placed in ice-cold saline and immediately mounted, via the ascending aorta, onto a perfusion apparatus. The hearts were perfused with a non-recirculating perfusate at a constant hydrostatic pressure of 75–80 cmH_2O (Langendorff preparation). The perfusate was a modified Krebs-Henseleit solution containing (in mM): NaCl 127, KCl 4.7, $MgSO_4$ 1.1, KH_2PO_4 1.2, glucose 5.5, pyruvate 2.0, $CaCl_2$ 2.5. The perfusate was equilibrated with 5% CO_2:95% O_2 at 37°C and the pH was adjusted with $NaHCO_3$ (20–25 mM) to 7.40. The perfusate was filtered (0.45 μm) before use. The perfusate in the reservoir was continuously pumped through a 3 μm filter to prevent particulate matter from entering the coronary circulation. The pericardium and adherent lung tissue were removed. The pulmonary artery was cannulated to allow coronary venous drainage. A saline-filled catheter was attached at one end to a pressure transducer (Gould P23ID)

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and at the other to a balloon which was inserted into the left ventricle for the measurement of left ventricular (LV) pressure (P). The balloon was inflated to achieve an end-diastolic LVP of 5–10 mmHg. Heart rate and LV *dP/dt* were obtained via electronic differentiation from the pressure signal using a biotachometer (Grass 7P411) and analog differentiation amplifier (Grass 7P20C), respectively. Hearts were paced at 275–285 beats min^{-1} (Grass Model S44 Stimulator, Stimulus Isolation Unit SIU5A, and Constant Current Unit CCU1A) via two Teflon-coated stainless steel wires inserted into the right ventricular myocardium. Coronary flow was measured with a square wave electromagnetic flowmeter (Carolina) and a cannulating transducer placed in the aortic inflow line. All meters and transducers were calibrated daily. Coronary flow, heart rate, LVP and LV *dP/dt* were recorded continuously on a Grass Model 7E Polygraph. A 30 min equilibration period was allowed before the experiment was begun.

Vasodilator drugs were given by intra-arterial bolus injection (0.25 ml followed by a 0.5 ml flush with arterial perfusate) through a stopcock-controlled injection port above the flow transducer. Inhibitors were infused (Harvard Infusion Pump, Model 22) intra-arterially at 1/50 the total inflow rate through an infusion port in the flow transducer.

Preparations were not used for experiments if the spontaneous (unpaced) heart rate was less than 190 beats min^{-1}, if baseline coronary flow was less than 3 ml min^{-1} g^{-1} or if coronary flow failed to increase by at least 100% following a 30-s period of inflow occlusion. All experiments were completed in less than 3 h. Thirty-second occlusions were performed periodically throughout each experiment to verify the capacity of the preparation to vasodilate.

Responses to vasodilator drug injections were measured first under control conditions, and again during infusion of one of the inhibiting agents used in this study. Thus each heart served as its own control. During the control run, several doses of each vasodilator were tested. A dose of each vasodilator agent was chosen that produced a response equivalent to 50–75% of the maximum flow response to that drug in each heart. This dose was used as the challenge dose for the remainder of the experiment. There were small variations among the hearts within an experimental series in the dose of the vasodilator agent chosen as the challenge dose by this criterion. The range of doses used as the challenge in each series is given in the text. Maximal coronary flow was measured as the peak flow following release of a 30 s inflow occlusion.

Drugs

Acetylcholine, sodium nitroprusside and papaverine (Sigma) and ATP and adenosine (Boehringer-Mannheim) were dissolved in 0.9% NaCl. Nitro-L-arginine (Sigma) was dissolved in 0.9% NaCl by sonication for 40–60 min at 30°C. Arachidonic acid (Nu-Chek, 10 mg ml^{-1}) was dissolved in ethanol, and aliquots of this stock were diluted 1:10 with 100 mM Na_2HPO_4 and further diluted with 0.9% NaCl to achieve the injectate concentration. Indomethacin (Merck Sharpe & Dohme, 1 mg ml^{-1}) was dissolved in 100 mM Na_2CO_3 (pH 7.4–7.5) and diluted with 0.9% NaCl (final pH 7.1–7.2). PGI_2 (Upjohn, 50 μg) was dissolved in 1 ml of Tris buffer (100 mM, pH 8) and diluted with 0.9% NaCl. XAC (Research Biochemicals) was dissolved in 0.1 M NaOH, then diluted 1:100 with 0.9% NaCl.

Statistics

Baseline and peak flow responses to each vasodilator, before and during infusion of the test agent, were evaluated by analysis of variance (ANOVA), using a model that accounted for repeated measurements in an individual heart. *Post-hoc* intergroup analysis was performed using the F-test of Sokal & Rohlf (1969). A $P < 0.05$ was taken as indicating statistical significance. Means ± 1 s.e.mean are given in the text and figures.

Use of animals

All procedures used in this study that were related to the use and care of laboratory animals were reviewed and approved in advance by the Institutional Animal Care and Use Committee (IACUC) of New York Medical College, according to the guidelines set forth in the U.S. National Institutes of Health Guide for the Care and Use of Laboratory Animals. Moreover, all experiments were conducted in accord with the Guiding Principles in the Care and Use of Animals as approved by the Council of the American Physiological Society.

Results

Effects of nitro-L-arginine

Under control conditions, acetylcholine induced a 2.6 ± 0.5 ml min^{-1} g^{-1} increase in coronary flow from a baseline of 6.2 ± 0.03 ml min^{-1} g^{-1}. During nitro-L-arginine (NLA) infusion (500 μM) the acetylcholine-induced coronary dilatation was completely abolished. Most of the hearts (10 of 14), in fact, displayed a vasoconstriction response to acetylcholine (−0.7 ± 0.4 ml min^{-1} g^{-1}) during NLA (Figure 1). This indicates the successful blockade by NLA of the NO synthesis that mediates the dilator action of acetylcholine. Smaller concentrations of NLA (50–200 μM) were found in pilot experiments to attenuate, but not abolish, the coronary dilator effect of acetylcholine. This inhibition was specific in that coronary flow responses to adenosine and papaverine were not inhibited by NLA (Figure 1). ATP-induced coronary dilatation was not affected by NLA (Figure 1). Coronary dilatation in response to sodium nitroprusside was actually increased by 46 ± 11% over the control response ($P < 0.05$).

Effects of indomethacin

Arachidonic acid produced a biphasic coronary response, an initial transient vasoconstriction followed by vasodilatation, which lasted several minutes. As shown in Figures 2 and 3, indomethacin (2.8 μM) completely eliminated the vasoconstriction phase of the arachidonic acid response. The peak flow in the vasodilatation phase of the response was reduced by 71 ± 13% (flow increase of 0.5 ± 0.2 ml min^{-1} g^{-1} vs. control response of 2.0 ± 0.4 ml min^{-1} g^{-1}, $P < 0.05$, Figure 3) and the duration of this phase was greatly shortened (Figure 2). Indomethacin did not affect adenosine or PGI_2-induced vasodilatations, indicating the specificity of its action as a cyclooxygenase inhibitor. ATP-induced coronary dilatation

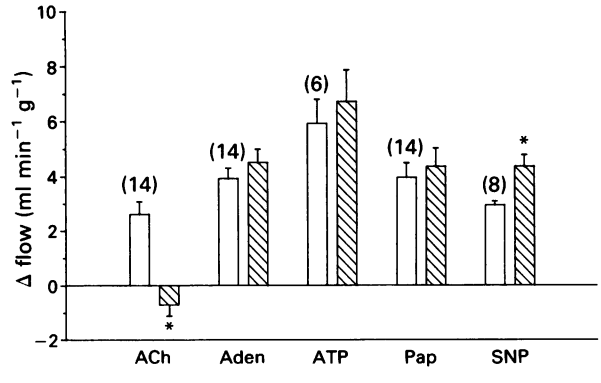

Figure 1 Peak change in coronary flow (ml min^{-1} g^{-1}) following bolus injection of acetylcholine (ACh, 2.5–7.5 nmol), adenosine (Aden, 25–75 nmol), ATP (25 nmol), papaverine (Pap, 25–75 nmol) or sodium nitroprusside (SNP, 25 nmol) before (open columns) and during nitro-L-arginine (NLA, 500 μM) infusion (hatched columns). The number of hearts is indicated in parentheses. * $P < 0.05$ vs. control (pre-NLA infusion). Each error bar = 1 s.e.mean.

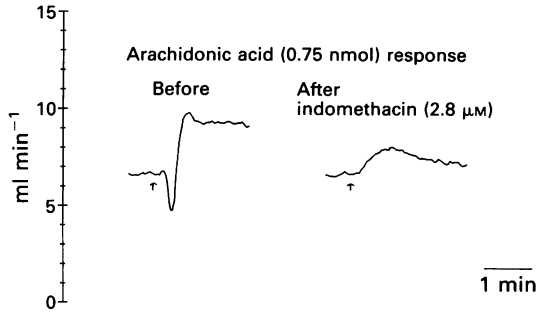

Figure 2 A representative biphasic response of coronary flow in the guinea-pig isolated heart to bolus injection of arachidonic acid (0.75 nmol, AA) before and during indomethacin (2.8 μM) infusion. ↑ = AA injection; $n = 7$ hearts. Note that in the control response (before), flow has returned only slightly towards control by the end of the tracing depicted whereas the post-indomethacin response is virtually complete within the same time frame.

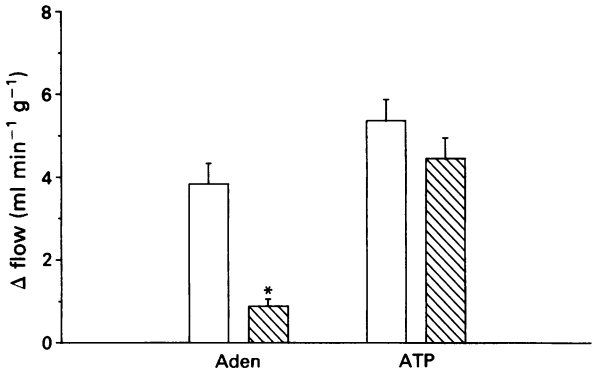

Figure 4 Peak change in coronary flow in response to adenosine (Aden, 25–75 nmol) and ATP (25 nmol) before (control, open columns) and during xanthine amine congener infusion (2 μM, hatched columns). $n = 7$ hearts; * $P < 0.05$ vs. control.

responses, however, were reduced 31 ± 4% during indomethacin infusion (control response of 3.9 ± 0.3 ml min^{-1} g^{-1} vs. 2.7 ± 0.2 ml min^{-1} g^{-1} during indomethacin, $P < 0.05$, Figure 3). Infusion of the indomethacin vehicle (5 mM Na_2CO_3 in isotonic saline) had no effect on baseline flow or any of the flow responses to the vasodilators used in this study.

Effects of xanthine amine congener

XAC, a potent adenosine receptor blocker, was infused at a concentration of 2 μM. At this concentration, XAC inhibited adenosine-induced coronary vasodilatation by 75 ± 5% (flow response of 0.9 ± 0.2 ml min^{-1} g^{-1} during XAC infusion vs. control response of 3.8 ± 0.5 ml min^{-1} g^{-1}, $P < 0.05$, Figure 4). ATP responses, however, were not significantly changed (4.5 ± 0.5 ml min^{-1} g^{-1} vs. control response of 5.4 ± 0.5 ml min^{-1} g^{-1}). The XAC vehicle (1 mM NaOH in isotonic saline), had no effect on baseline flow or any of the flow responses.

Effects of inhibitors on baseline coronary flow

The inhibitor drugs used in this study each had a slight, but statistically significant, effect on baseline coronary flow. As shown in Figure 5, NLA (500 μM) decreased baseline flow by 16 ± 0.8% from 6.2 ± 0.03 to 5.2 ± 0.05 ml min^{-1} g^{-1} ($n = 14$ hearts, $P < 0.05$). XAC (2 μM) decreased baseline flow by 25 ± 0.7% from 5.2 ± 0.05 to 3.9 ± 0.07 ml min^{-1} g^{-1} ($n = 7$ hearts, $P < 0.05$). Indomethacin (2.8 μM) caused a slight increase in baseline flow, by 8 ± 1% from 6.2 ± 0.2 to 6.7 ± 0.2 ml min^{-1} g^{-1} ($n = 7$ hearts, $P < 0.05$).

Discussion

The major conclusions reached in this study are that (1) ATP is a coronary vasodilator in the guinea-pig isolated, perfused heart; (2) nitric oxide (NO) and adenosine appear to exert a basal influence on the resting coronary flow in this preparation; (3) ATP-induced coronary vasodilatation in the guinea-pig isolated heart is not dependent on NO; (4) ATP-induced coronary vasodilatation is partially (about one-third) due to prostaglandin production; and (5) ATP-induced coronary vasodilatation is not due to the degradation of ATP to adenosine, but rather to the direct action of ATP, presumably, on specific ATP receptors.

It has been demonstrated that the vasodilator action of ATP in a variety of experimental preparations is at least partly dependent upon the endothelium (De Mey & Vanhoutte, 1981; Cassis *et al.*, 1987; Hopwood *et al.*, 1989; Lee *et al.*, 1990; Radermacher *et al.*, 1990; Koller *et al.*, 1991). Many

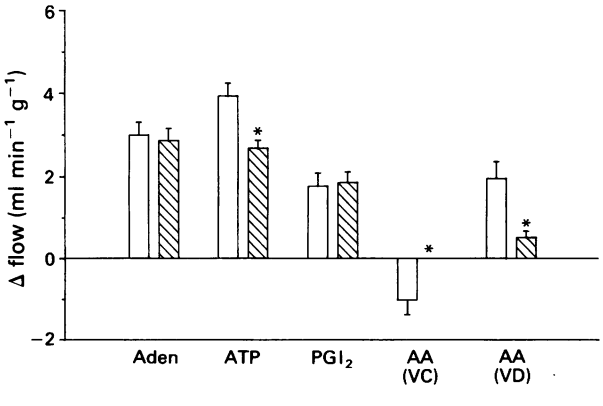

Figure 3 Peak change in coronary flow in response to bolus injections of adenosine (Aden, 7.5–25 nmol), ATP (7.5–25 nmol), prostacyclin (PGI_2, 3.35 nmol) and arachidonic acid (AA, 0.75 nmol) before (control, open columns) and during indomethacin infusion (2.8 μM, hatched columns). $n = 7$ hearts; * $P < 0.05$ vs. control; vasoconstrictor (VC) and vasodilator (VD) phases of arachidonic acid response are depicted separately.

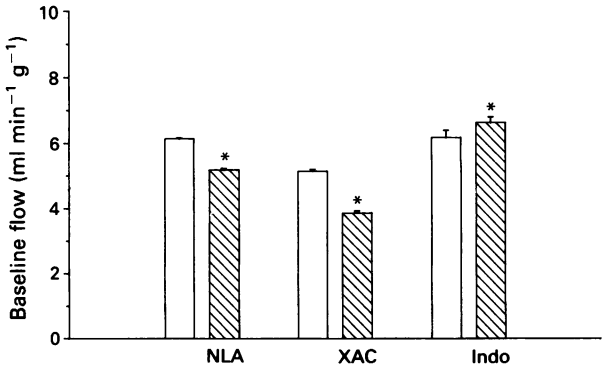

Figure 5 Baseline coronary flow before infusion (control, open columns) and during infusion of nitro-L-arginine (NLA, 500 μM, $n = 14$ hearts), xanthine amine congener (XAC, 2 μM, $n = 7$ hearts) or indomethacin (Indo, 2.8 μM, $n = 7$ hearts) (hatched columns). * $P < 0.05$ vs. control.

isolated conduit blood vessels show a diminished relaxation response to ATP after the endothelium has been physically or chemically removed (De Mey & Vanhoutte, 1981; De Mey et al., 1982; Cassis et al., 1987). Because it is difficult to damage the endothelium selectively in intact vascular beds, the endothelium-dependence of the microvascular vasodilator action of ATP is less well established.

In the perfused rabbit hindlimb, the vasodilator effects of acetylcholine, ATP and substance P, but not those of adenosine, nitroglycerin or PGE_2 were reduced after gossypol (Pohl et al., 1987; Dezsi et al., 1990). Although gossypol was regarded in these studies as an inhibitor of endothelium-mediated vasodilatation, this drug may have other actions (Ye et al., 1987). Therefore, the implication of these studies that ATP is an endothelium-dependent or NO-dependent dilator in the rabbit hindlimb remains unproven. Koller et al. (1991), using a light-dye method to create focal endothelial damage, have demonstrated the endothelium-dependence of ATP-induced arteriolar dilatation in the rat cremaster muscle microcirculation without defining the specific mediator involved.

No comparably definitive study has been reported for the coronary circulation, although several studies have suggested the involvement of an endothelium-derived relaxing factor (EDRF) in the coronary dilator action of ATP. For example, coronary dilator responses to ATP were attenuated by electrical stimulation of the perfusate (Lee et al., 1990) and by hydroquinone (Hopwood et al., 1989). Both electrical current and hydroquinone generate superoxide anions, which may then inactivate NO. It is possible, however, that these oxygen radicals might attenuate vasodilatation in some way other than by attacking NO. Thus, although there is suggestive evidence that ATP acts via the endothelium to cause vasodilatation in the coronary and skeletal muscle microcirculations, the identity of the endothelium-derived mediator is not certain. Despite these suggestive results, our finding that NLA fails to attenuate the coronary dilator action of ATP allows us to reject the hypothesis that NO is the endothelium-derived mediator of this response. NLA is superior to hydroquinone or electrolytic generation of oxygen radicals for use as a test of this hypothesis because, unlike oxygen radicals, arginine analogues such as NLA specifically inhibit NO synthesis (Palmer et al., 1988; Moore et al., 1990). Our finding that the dilator effect of acetylcholine is abolished, whereas the coronary responses to adenosine and papaverine are preserved, further supports the specificity of the action of NLA.

A number of recent studies support our present conclusion that NO may have little or nothing to do with ATP-induced vasodilatations in many vasculator beds. In studies using guinea-pig pulmonary artery (Sata et al., 1990), and isolated, perfused rabbit (Pohl et al., 1991) and guinea-pig (Kelm & Schrader, 1990) hearts, dilator actions of acetylcholine and ATP were differentially affected by a variety of agents, including a spin-trapping agent, NLA and oxyhaemoglobin. Since the vasorelaxant or vasodilator effects of acetylcholine were blocked by these agents whereas those of ATP were not, these studies suggest that mediators other than NO are involved in ATP-induced vasorelaxation in these preparations. In contrast, NO synthase inhibitors antagonized the rabbit hepatic arterial vascular responses to both ATP and acetylcholine in a recent study (Mathie et al., 1991), indicating that the mechanisms of ATP-induced vasodilatation are tissue-specific.

It also remains possible that NO may interact synergistically with prostaglandins or other vasodilator metabolites and thus contribute to the coronary dilator response to ATP. Such synergism must be either small or compensated for when absent however, because blockade of NO synthase had no apparent antagonistic effect on ATP-induced coronary dilatation in our experiments.

In our study, the responses to SNP were potentiated by NLA, in keeping with other reports (Busse et al., 1989; Mathie et al., 1991; Moncada et al., 1991; Mugge et al., 1991). It is possible, that basal release of NO may slightly diminish the vasodilator reserve of the coronary bed, thus decreasing the responses elicited by superimposed vasodilator stimuli. However, this effect may be specific for interactions with guanylate cyclase (Moncada et al., 1991), rather than just a general baseline effect, because flow responses to other dilator agents (adenosine, papaverine) were not significantly potentiated.

ATP stimulates the production of a vasodilator prostaglandin (PGI_2) from the heart (Minkes et al., 1973; Needleman et al., 1974) and from cultured vascular endothelial cells (Needham et al., 1987). In our experiments with guinea-pig hearts, arachidonic acid produced a biphasic response in agreement with previous observations (Talesnik, 1986). Indomethacin, which is a specific inhibitor of cyclo-oxygenase, inhibits the biosynthesis of both vasodilator and vasoconstrictor prostaglandins and thromboxanes. Our indomethacin dose greatly attenuated both vasoconstrictor and vasodilator phases of the arachidonic acid response. ATP-induced vasodilatation, however, was reduced by less than one-third. Therefore, while vasodilator prostaglandins, most likely PGI_2, are involved in the mediation of the coronary response to ATP in the guinea-pig isolated heart, they do not provide a full explanation of the vascular response. In previous work, indomethacin greatly attenuated ATP-induced coronary dilatation in some studies (Minkes et al., 1973; Needleman et al., 1974), but not in others (Lee et al., 1990; Pohl et al., 1991). This discrepancy could be due in part to species differences and to differences in the physiological status of the various preparations. A prior study of guinea-pig heart found no effect of indomethacin on the coronary dilator action of ATP, but that study used a K^+-arrested heart (Lee et al., 1990). In the present study, in contrast, we used a beating, pressure developing heart preparation.

It was also possible that ATP-induced coronary dilatation might be mediated by adenosine formed from the rapid degradation of ATP by ectoenzymes on both endothelium and vascular smooth muscle (Pearson et al., 1980; Moody et al., 1984). Indeed, Pohl et al. (1991) found that less than 5% of the 1 μM ATP infused into rabbit isolated hearts appeared in the coronary effluent after one pass through the heart. We found that the potent adenosine receptor antagonist, XAC, had no significant effect on ATP-induced dilator responses despite the fact that XAC attenuated adenosine-induced vasodilator responses by more than 75%. Therefore, degradation of ATP to adenosine was not responsible for the observed increases in coronary flow produced by ATP.

Baseline coronary flow was reduced by NLA, as has been found previously not only for the heart, but also for other vascular beds (Amezcua et al., 1989; Kelm & Schrader, 1990; Moore et al., 1990; Radermacher et al., 1990; Mugge et al., 1991). This suggests that in the guinea-pig isolated heart, there is significant basal production of NO and further suggests that NO contributes a dilator influence in the regulation of basal vasomotor tone and vascular resistance in this preparation.

In a similar fashion, XAC reduced baseline coronary flow, thus implying a basal release of adenosine, which would contribute to the regulation of basal vascular resistance in this preparation. Wei et al. (1988) also found consistent and reproducible reductions in basal coronary flow in this same preparation after infusion of adenosine deaminase, the enzyme that converts adenosine to vasoinactive inosine. In contrast, Kroll & Feigl (1985) found no decrease in basal coronary following adenosine deaminase infusion. Thus the importance of adenosine in the regulation of basal coronary blood flow might be species-dependent.

Baseline flow changes with indomethacin administration seem to be variable in the literature. Small increases (Sunahara & Talesnik, 1979; present study), no change (Harlan et al., 1978; Lee et al., 1990) or small decreases (Hintze & Kaley, 1977) in basal coronary flow or vascular conductance with indomethacin have been described.

The changes in baseline flow with these inhibitors did not affect the conclusions of the present study as the changes in

baseline flow were small. Moreover, the inhibitions of the vasodilator responses to acetylcholine by NLA, to adenosine by XAC, and to arachidonic acid by indomethacin were specific in that the vasodilator responses to other dilator agents were not affected. Therefore, non-specific changes in the responses due to baseline flow changes may be ruled out.

The question that is raised by this study is, if NO and adenosine do not contribute to ATP-induced vasodilatation and prostaglandins (PGI_2) mediate less than one-third of ATP-induced vasodilatation, what factor(s) do provide for most of the coronary vasodilator response produced by ATP?

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